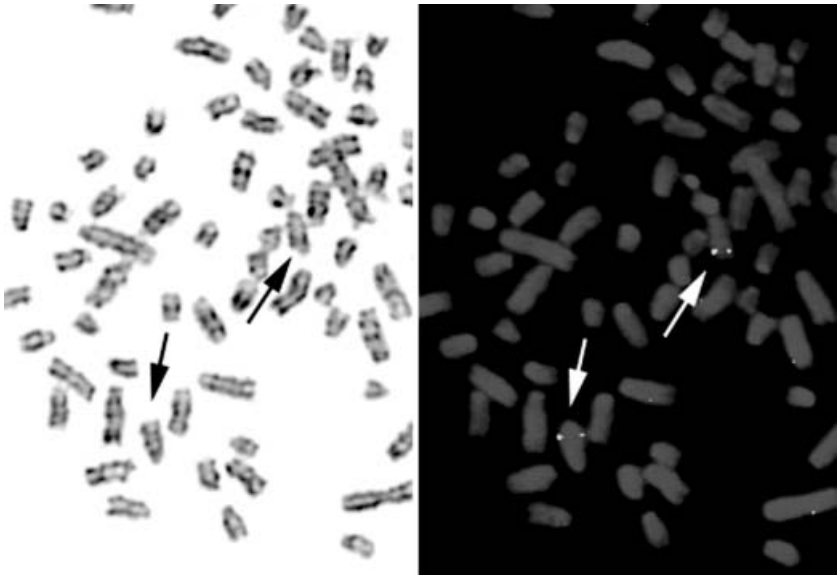

Figure 1 Metaphase spread after fluorescence *in situ* hybridization showing signals on both chromosomes 17 (right) and the same metaphase after GTG-banding (left).

BAC library screening: In order to isolate a FISH probe, the DogBAC canine BAC library[6] (http://www.dogmap.ch/) was polymerase chain reaction (PCR)-screened. Primers were designed using canine mRNA sequence GenBank accession no. U62093 (primer UP: GACTGAGTACAAACTGGTGG and primer LO: GGGCCTCACCTCTATGGTG). The PCR conditions were established on canine blood genomic DNA, the corresponding PCR product cloned and verified by sequencing. The positive BAC clone (DogBAC library ID S050P24H09) was verified by PCR and sequencing.

Gene mapping: For mapping the chromosomal location of the canine *NRAS* gene, metaphase preparations and FISH were performed as described previously.[7] Ten well spread metaphases exhibited a signal on CFA 17 on both chromatids of both chromosomes (Fig. 1), following the nomenclature of the canine karyotype as established by Reimann *et al.*[8]

Comments: NRAS mutations in humans have been found in 30% of liver tumours, 40% of myelodysplastic syndrome, 30% of acute myelogenous leukaemia, 13% of brain tumours and in 53% of follicular and 60% of undifferentiated papillary thyroid tumours.[9] In dogs, depending on tumour type, comparable occurrences exist in malignant melanomas,[5] while fibrosarcomas showed no amino acid alteration of the NRAS protein (H. Murua Escobar, K. Günther, A. Richter, J. T. Soller, S. Winkler, I. Nolte & J. Bullerdiek 2004, personal communication). Overall, data available on involvement of *ras* proto-oncogenes in tumours of dogs are still insufficient. Knowledge of the cytogenetic properties of *NRAS* will further the understanding of this important gene. The mapping results obtained in this study are in accordance with the known homology between canine chromosome 17 and the centromer-proximal regions 11.1–13.3 of the p-arm of human chromosome 1.[10]

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Linkage mapping of chicken *ovoinhibitor* and *ovomucoid* genes to chromosome 13

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Source/description: Ovoinhibitor (OIH) and ovomucoid (OVM) are the major proteinase inhibitors constituting 1.5 and 11% of the total proteins in hen egg white, respectively. Although OVM exerts its antiprotease activity only against trypsin, OIH has a wide spectrum of inhibitory activity for other proteinases that occur in chicken egg white and blood plasma.[1] They are functionally similar proteins and having multiple domains with a characteristic pattern of disulphide bridges.[1] From the analysis of DNA sequences and the positions of exons and introns, it

is thought that OVM and OIH evolved from a common primordial single-domain inhibitor.[2] Furthermore, it has been proposal that *OVM* will be localized on chicken chromosomes 10–15, based on chromosome fractionation.[3] However, their exact chromosomal locations are yet to be clarified. Therefore, in this study we performed the mapping of chicken *OIH* and *OVM* to specific chromosomes.

For this study, primers were designed to amplify the region corresponding to intron 15–exon 16 region of the chicken *OIH* gene (GenBank accession nos. AY225161 and AH002465): *OIH* – forward: 5′-TAGTTTGTCCCACAAGCTCATCAG-3′, *OIH* – reverse: 5′-TTAACACGCTGCCATACGCACGAG-3′) and exon 1–intron 1 region of the chicken *OVM* gene (GenBank accession nos. J00902 and AB164053): *OVM* – forward: 5′-ATCTCAGGAGCAGAGCACCGGCAG-3′, *OVM* – reverse: 5′-GCAGTGTAACAGAGCTGAGAGCGT-3′.

PCR reaction: The polymerase chain reactions (PCR) contained 40 ng of genomic DNA, PCR buffer containing 2 mM $MgCl_2$, 200 μM of each dNTP, 10 μM of each primer and 1 U Ex *Taq*DNA polymerase (Takara, Otsu, Japan) in a final volume of 20 μl. The PCR reactions were performed on a GeneAmp PCR system 9700 (Applied Biosystems, Tokyo, Japan) with the following profile: *OIH* – initial denaturation of 3 min at 94 °C, 35 cycles of 94 °C for 30 s, 58 °C for 30 s and 72 °C for 40 s, and a final elongation step of 7 min at 72 °C; *OVM*: initial denaturation of 3 min at 94 °C, 35 cycles of 94 °C for 30 s, 65 °C for 30 s and 72 °C for 40 s, and a final elongation step of 7 min at 72 °C. Digestion of 5 μl of *OIH* PCR product was performed with 1 U *Bsr*I at 37 °C overnight. Digestion of 5 μl of *OVM* PCR product was performed with 1 U *Hpy188*at 37 °C overnight. Nucleotide sequences were assigned GenBank accession nos. AY225161 and AB164053 for *OIH* and *OVM*, respectively.

Polymorphisms and genotyping: Genomic DNA extracted from the Kobe University (KU) resource family [4] (♂: White Leghorn, ♀: Fayoumi, F_1: White Leghorn × Fayoumi) and the East Lansing reference population [5] (♂: Red Jungle Fowl, ♀: White Leghorn, F_1: Red Jungle Fowl × White Leghorn) were used as templates for *OIH* and *OVM* PCR. Sequence comparison of intron 15-exon 16 of the chicken *OIH* gene in the KU resource population revealed two single nucleotide polymorphisms (SNPs), corresponding to nucleotides 328 and 438 (AY225161; transitions C/T and G/A, respectively). No polymorphism was found in the East Lansing reference population. Digestion of the PCR fragment with *Bsr*I resolved the SNP at position 438, resulting in two alleles (Fig. 1a): allele *A* (fragment 286 bp; uncut) and allele *B* (175 + 111 bp).

Sequence comparison of the exon 1–intron 1 of the chicken *OVM* gene revealed two polymorphisms in the East Lansing reference population corresponding to nucleotides 61 and 115–116 (AB164053; transitions C/T and TT/GG, respectively). The SNP at position 61 can be detected by PCR-restriction fragment length polymorphism (RFLP) using digestion of the PCR amplicon with *Hpy188*I. As shown in Fig. 1b, two alleles were detected: allele *C* (fragment 136 + 125 + 67 bp) and allele *D* (192 + 136 bp).

Linkage analysis and chromosomal localization: Segregation of the *OIH* PCR-RFLP was analysed for linkage with 800 genetic markers previously genotyped in the KU reference population

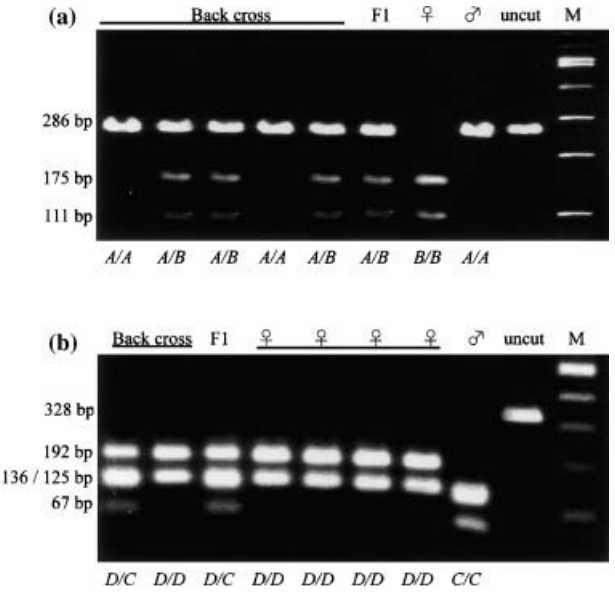

Figure 1 (a) Agarose gel electrophoresis (2.5%) showing genotypes of the chicken *OIH* gene after digestion of PCR products with *Bsr*I. ♂: White Leghorn, ♀: Fayoumi, F_1: White Leghorn × Fayoumi, Back cross: F1♀ × White Leghorn ♂. (b) Agarose gel electrophoresis (2.5%) showing genotypes of the chicken *OVM* gene after digestion of PCR products with *Hpy188*I. M: 2000–100 bp ladder marker (Toyobo, Japan). ♂: Red jungle fowl, ♀: White Leghorn, F_1: Red jungle fowl × White Leghorn, Back cross: F1♂ × White Leghorn ♀.

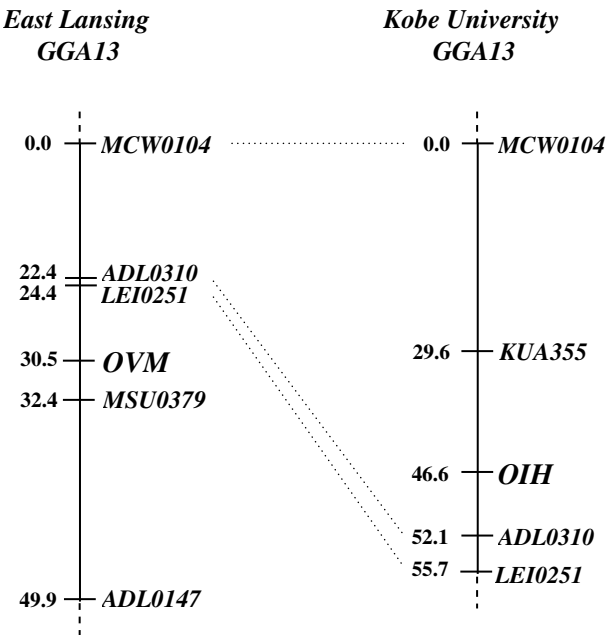

Figure 2 Comparative maps of chicken chromosome 13 using the Kobe University resource family (KU) and the East Lansing reference population (EL). Distances are indicated in centiMorgan (cM). For simplicity, not all markers on the two maps are shown.

using the method reported by Lee *et al* (2002).[4] *OIH* was significantly linked to two genetic markers located on the chromosome 13: *ADL0310* and *KUA355* (LOD = 11.5 and 5.9, respectively) (Fig. 2). Genetic distances were (*ADL03 10*) – 5.5 cM – (*OIH*) – 17.0 cM – (*KUA355*). Segregation of the *OVM* PCR-RFLP was compared with 1257 genetic markers previously genotyped on the East Lansing reference population. Linkage analysis was performed according to Crittenden *et al.* (1992).[5] The *OVM* marker was significantly linked to two genetic markers located on chromosome 13: *MSU0370* and *LEI0251*, with LOD 13.5 and 9.8, respectively. Genetic distances were (*MSU0379*) – 1.9 cM (*OVM*) – 6.1 cM – (*LEI0251*) (Fig. 2).

Comments: Twenty genes whose orthologues are all located on HSA5 (5q23 – 5q35) have been mapped on chicken chromosome 13 (GGA13).[6] However, orthologous genes corresponding to *OIH* and *OVM* are yet to be identified in humans, but comparative analysis of exons and introns has suggested that chicken *OIH* and *OVM* genes are associated with the human pancreatic secretory trypsin inhibitor (*PSTI*) gene.[7] Thus, the human *PSTI* gene, found on HSA5, may be the orthologous gene corresponding to the chicken *OIH* and/or *OVM* genes on GGA13, but further investigation is needed.

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Cloning and mapping of canine *KIAA1753*

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Description: Progressive rod-cone degeneration (*prcd*) is one of several forms of progressive retinal atrophy (PRA), a group of inherited retinal diseases that are the canine equivalent of human retinitis pigmentosa (RP)[1, 2]. Based on phenotypic similarities and map position, *prcd* has been proposed as the locus homologue for the RP17, and is the only animal model for human RP17 form of RP[3]. Our laboratory has mapped the *prcd* gene locus to the short arm of canine chromosome 9 (CFA9) in a large genomic area in which the closest telomeric marker is approximately 6 cM away, and no centromeric marker has been identified[4]. *KIAA1753* is a gene of unknown function that has been cloned among a non-redundant set of more than 1100 genes expressed in human retinal pigment epithelium (RPE)[5]. In human, *KIAA1753* maps to the HSA17q25.3 region of HSA17qter, a region of synteny with the centromeric end of CFA9.

Table 1 Sequences and positions of the primers designed from canine[1] or human[2] sequences to clone canine *KIAA1753* cDNA. The sizes of the PCR products for each pair of primers is also indicated.

Primer	Sequence (5′-3′)	Location	Designed from	Amplicon
K35F	GAAGAGGCAGGAAGACAGG	1–19	BAC canine sequences[1]	
K34R	CTTGGTGCAGTAGACGTCA	280-298	BAC canine sequences	298 bp
K39F	AGAAACCGCAGCACTACAC	108-126	BAC canine sequences	
K34R	CTTGGTGCAGTAGACGTCA	280-298	BAC canine sequences	190 bp
K7F	AGTGCCCACTCTTTGTGCA	153-171	BAC canine sequences	
K8R	TGAAGGAGAGGCTGCTCTT	1373-1391	BAC canine sequences	1238 bp
K21F	TCCTGGCTTTGAGAGGGAA	1275-1293	BAC canine sequences	
K23R	CCTATGACTGACTCTACCG	1559-1577	BAC canine sequences	302 bp
K40F	GTCCCTGGCATGAATGCAA	1510-1528	BAC canine sequences	
K29R	CACTGCTTGATGGTGCCAT	2005-2023	BAC canine sequences	513 bp
K28F	TTGCCTCTGGTAGCTTCTC	1904-1922	BAC canine sequences	
K31R	GCTCCTGACACTTAAGGCA	2335-2353	BAC canine sequences	449 bp
Kh17F	TGCCTTAAGTGTCAGGAGC	2327–2345	Human sequences[2]	
Kh19R	CCAAGGAATGCAAACGGAG	3398–3416	human sequences	1089 bp
KIAA1753F	TGGCAGGGGATGTGTTTCT	2790–2808	BAC canine sequences	
KIAA1753R	CCAAGGAATGCATACGGAG	3398–3416	BAC canine sequences	627 bp

[1]Canine BAC clone 298M24.
[2]GenBank no. XM_036115.